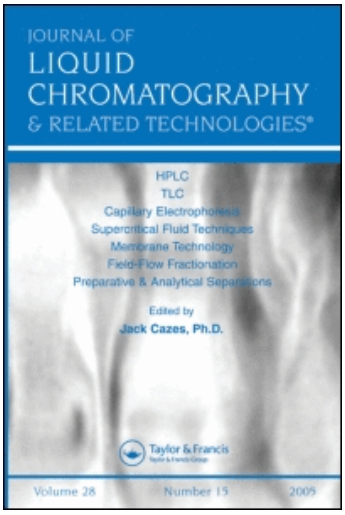


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# The Determination of Iodine in Milk and Milk Chocolate by Anion HPLC

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## ABSTRACT

An HPLC method is described for the extraction and analysis of iodine as iodide in milk, cocoa beans and milk chocolate. Prior to analysis samples are finely ground if necessary and combusted in a modified Shoeninger flask. The liberated halide is absorbed in a basic reducing medium which is concentrated and brought to volume with distilled water prior. HPLC analysis used an 8 mm 5μ $C_{18}$ cartridge in a RCM-100 with detection of the iodide ion at 226 nm. The mobile phase consisted of an ion-pairing agent, buffer and acetonitrile. The method is accurate and precise showing reasonable agreement with a National Bureau of Standards spray dried milk sample.

## INTRODUCTION

There is much interest in the analysis of iodide in various commodities, especially milk and milk products. With the use of iodophors as disinfectants in the milk industry some data indicates that there is a resulting increase in iodine consumption by the public, leading to a potential increase in thyroid disorders. There is also interest in endogenous iodine levels in various commodities.

Methods employed for the analysis in milk and other food include GC (1) ion-selective electrodes (2) microdistillation (3) and differential pulse polarography (4,5). Other methods proposed include neutron activation analysis (6), which while scientifically stimulating cannot be utilized in any but large laboratories. An AOAC



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Collaborative Study (7) with 7 samples and 8 laboratories using two methods showed a relative standard deviation of 77.9% between labs and 19.1% within labs. In that collaborative study the two methods used were the catalytic effect of iodine on the $Ce^{+4}$-$As^{+3}$ reaction and neutron activation analysis.

There has been much written about sample preparation for halide determination. A method which has been extremely useful is oxygen flask combustion. This method has seen wide use in the microdetermination of ions (8) and preliminary work on its use in iodine analysis was presented (9) but still required a complicated distillation before a final colorimetric determination step. The method proposed in this paper uses conventional HPLC equipment and a UV detector to accomplish the analysis of iodine in milk, cocoa beans and milk chocolate at lower levels of several hundred ppb. LC has been used for the analysis of various anions but has either used specialty packings (10) conductivity detectors (11) or has been done on relatively pure samples (12,13,14). Others have used various ion pairing agents but were limited to rather theoretical considerations (15,16,17).



## EXPERIMENTAL

### HPLC Apparatus

The HPLC system used consisted of a Model 6000A Solvent Delivery System (Waters Assoc.), a model 165 Variable Wavelength Detector operated at 226 nm (Beckman Inst.), a model 710B WISP Autoinjector, Model 720 System Controller and Model 730 Data Module (Waters Assoc.). The HPLC Column used was a 8 mm 5μ $C_{18}$ Cartridge for the RCM-100 (Waters Assoc.).

### HPLC Mobile Phase

45/25/25 (v/v/v) .0025m hexadecyltrimethylammoniumchloride/.05m $Na_2HPO_4$ /$CH_3CN$ at pH 6.8 ± 0.1 flowing at 2.5 ml/min.

## Iodide Standard

KI (alfa) dissolved in $CH_3OH$ for a final concentration of 0.01 g/µl. Check standard and prepare fresh when degradation occurs. Degradation can be seen by a decrease in peak length for the sample injection and the appearance of a peak in the chromatogram equivalent to the formation of $IO_3$.

## Other Reagents and Supplies

Other equipment used in this study consisted of a 5 liter oxygen combustion flask (9), rubber balloons, and a rotary evaporator. Other reagents were hydrazine monohydrate, KOH and oxygen.

## Preparation of Sample

If necessary, grind sample to particle size that allows it to pass through a 25 mesh sieve. For cocoa beans and chocolate weigh 0.5 g into Whatman 541 filter paper. For dry milk, use 0.25 g sample size. Place this into the sample holder of the combustion flask, containing 50 ml of distilled water, 50 µl of hydrazine monohydrate and 3.0 ml of 0.1M KOH and a stirring bar. Attach a rubber balloon to the side neck of the flask to allow expansion of the vapor in the flask. Fill the flask with oxygen, clamp the holder into place and ignite with a 650 watt photography lamp. Allow the sample to burn until the flame is extinguished and place the flask on a stirrer at high speed for at least 20 minutes or until the balloon is totally deflated. Transfer the solution into a 250 ml round bottom flask with three 25 ml portions of distilled water. Place on a rotary evaporate at 85-90°C and rotovap to dryness. Dilute to 2 ml with distilled water for the HPLC determination.

## HPLC Determination

Inject 50 to 100 µl of sample extract onto the column. After separation, calculate concentrations of iodide by comparing peak heights with peak heights of iodide standard solutions.

## RESULTS AND DISCUSSION

The potassium iodide standard was scanned from 190-700 nm using a Hitachi EPS-3T UV/Vis spectrophotometer. The potassium iodidi exhibited absorbance maximums at 196 and 226 nm. The use of the UV detection was based on earlier studies dealing with the UV spectra of inorganic anions (18). The HPLC standard was injected over a 12 hour period and no decay was evident. The standard was linear from 5 ng to 20 µg with a regression coefficient of 0.98. The 226 nm wavelength was chosen as the one of choice since the baseline was quietier than at 196 nm. Lower limits were 5 ng/inj and 100-150 ppb in cocoa beans. Precision studies were run on standard, cocoa bean and milk with data summarized in Table 1.

An evaluation of the method using KI standard (500 ppb) and filter paper was conducted and 97% recovery was achieved. Whatman 541 filter paper was primarily chosen due to its low ash.

A sample of nonfat dry milk was provided by NBS. The milk was reported to have an iodide concentration, for information use only, of 3.4 µg/g. Using a sample weight of 0.25 g, four analyses were



Table 1

Precision Study

| Sample | n | Conc | %Cv |
|---|---|---|---|
| Standard | 26 | 50 ng | 2.27 |
| Cocoa bean | 3 | 133.4 ng | 8.4 |
| Milk | 4 | 3.8 µg | 2.8 |

Table 2

Cocoa Bean Recovery Studies
n = 2

| Amt. Added | Amt. Recovered | % Recovery |
|---|---|---|
| +200 ng | 174 ng | 87 |
| +400 ng | 324 ng | 81 |

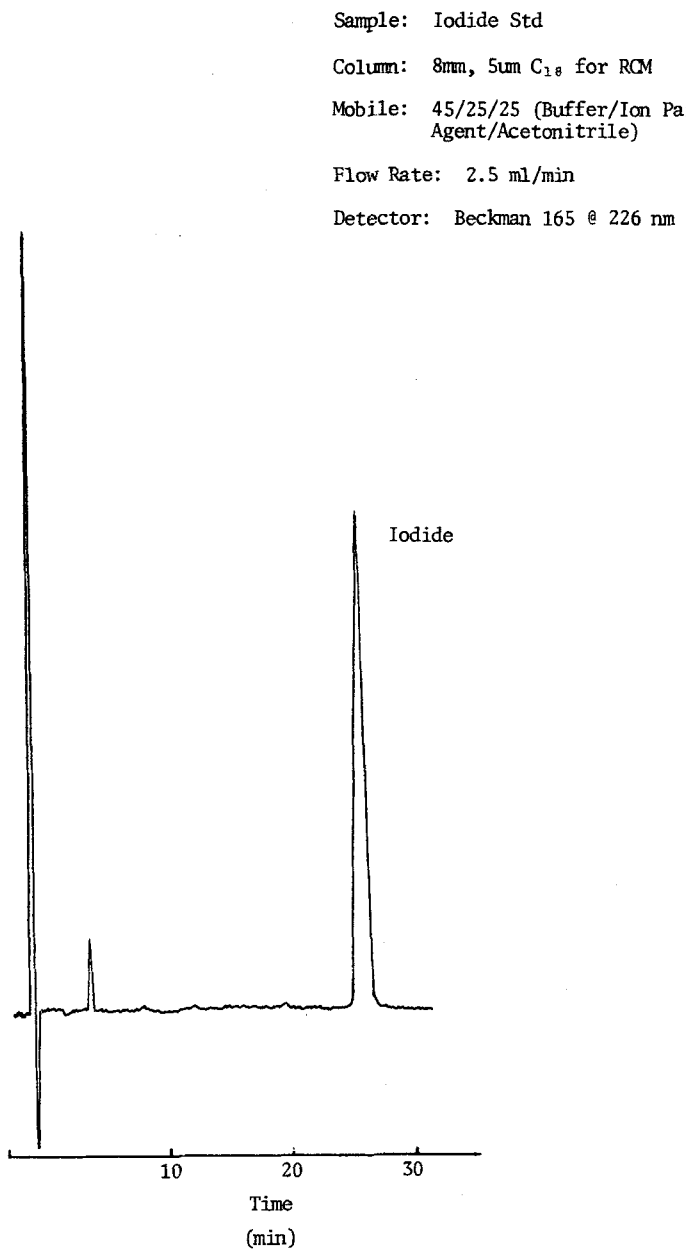


FIGURE 1 Chromatogram of Iodide Std.

Sample: Cocoa Bean

Column: 8mm, 5um $C_{18}$ for RCM

Mobile: 45/25/25 (Buffer/ Ion Pairing Agent/Acetonitrile)

Flow Rate: 2.5 ml/min

Detector: Beckman 165 @ 226 nm

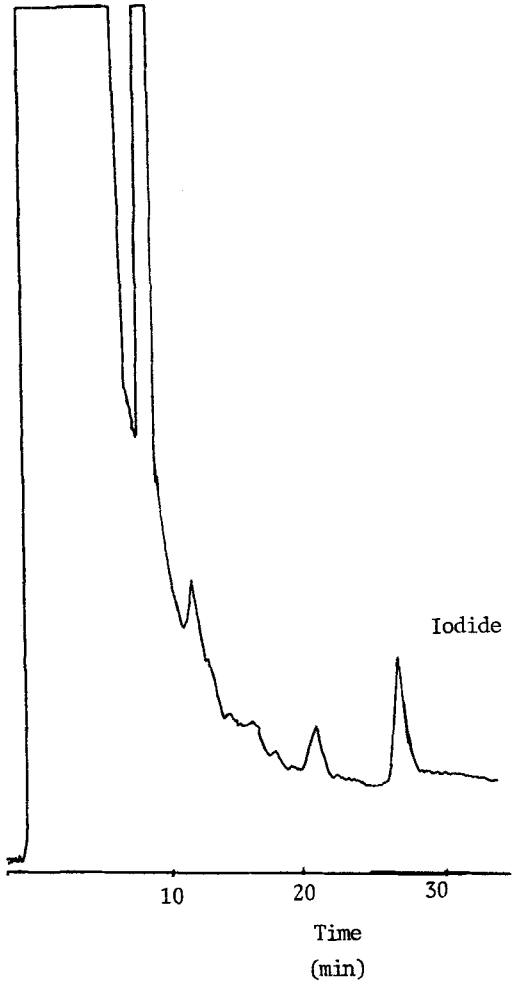


FIGURE 2 Chromatogram of Cocoa Bean Extract

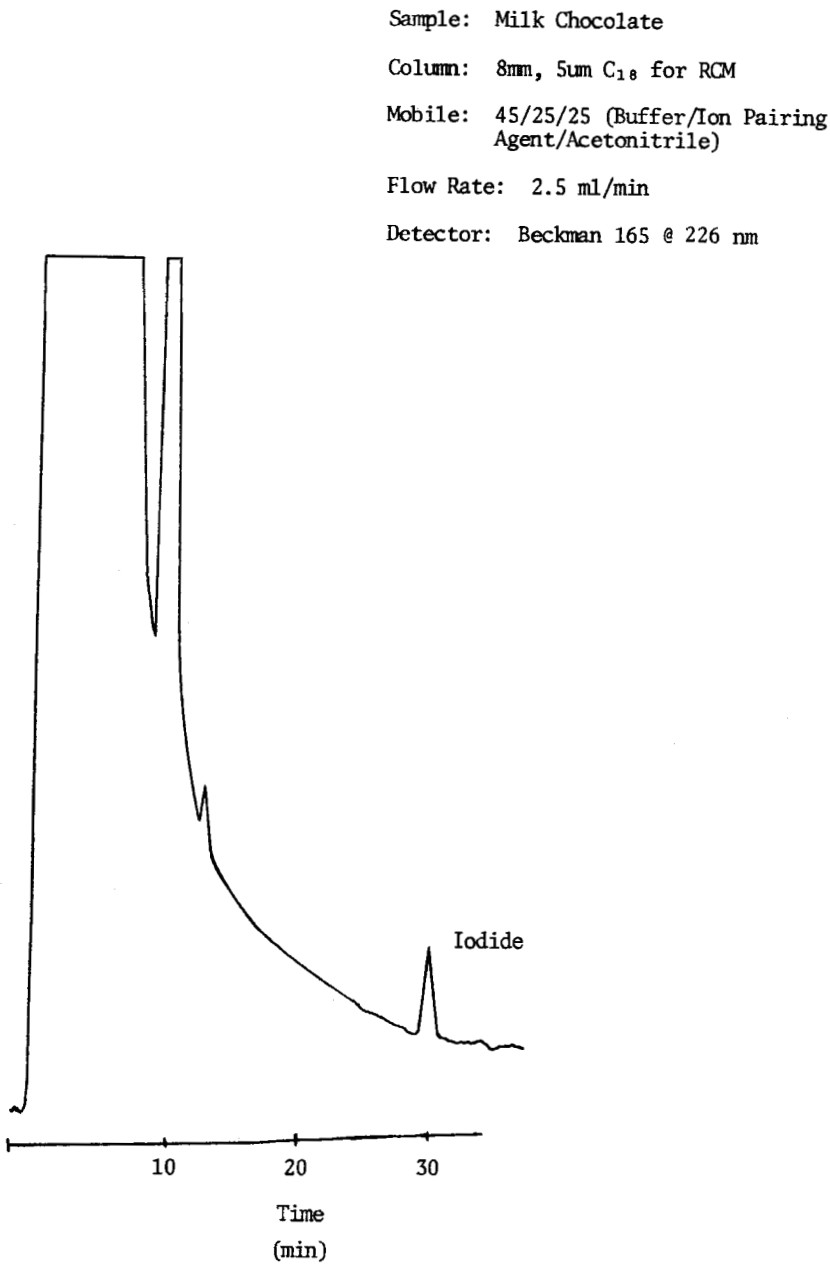


FIGURE 3 Chromatogram of Milk Chocolate Extract

Sample: Nonfat Dry Milk

Column: 8mm, 5um $C_{18}$ for RCM

Mobile: 45/25/25 (Buffer/Ion Pairing Agent/Acetonitrile)

Flow Rate: 2.5 ml/min

Detector: Beckman 165 @ 226 nm

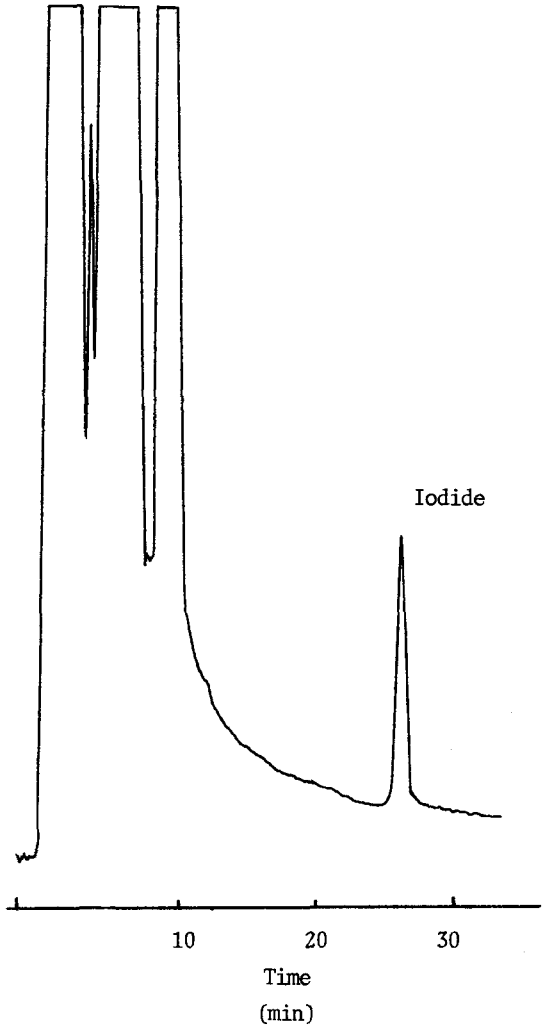


FIGURE 4 Chromatogram of Milk Extract

conducted with an average of 3.8 μg/g iodide. This sample was spiked at a 200 ng level (n = 2) and 95% recovery was achieved.

The method was evaluated on cocoa beans with recovery data summarized in Table 2.

Figures 1, 2, 3, and 4 show chromatograms of iodide standard, nonfat dry milk, cocoa bean and milk chocolate. All iodide peaks were scanned using the Model 165 detector. These scans were compared with scans including max of iodide standard to insure peak identity. The matrix of milk chocolate was analyzed with iodide concentration of 75 ppb for n = 2.

In summary, the method described is an accurate, precise and cost effective alternative for the analysis of iodide. The method proposed does not require the purchase of auxiliary HPLC equipment. It uses HPLC equipment found in many laboratories. The method uses accepted methods of halide determination for sample preparation prior to the final HPLC determination step. It is linear over a wide range with a lower limit of less than 100 ppb. Depending on sample matrix, much lower limits are potentially achievable. Additionally, work is continuing in our laboratory using post column reactions to increase lower limits. If an analyst uses the 190 - 200 nm region for detection of anions, then it is possible to detect fluoride and chloride. In the present mobile phase, these ions elute near void volume. Further work would therefore require adjustments in the mobile phase.



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